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Modulation of Hypothalamic Neuronal Activity through a Novel G-Protein Coupled Estrogen Membrane Receptor

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Abstract

Estrogens are involved in the hypothalamic control of multiple homeostatic functions including reproduction, stress responses, energy metabolism, sleep cycles, temperature regulation, and motivated behaviors. The actions of 17 β -estradiol (E₂) in the brain have been attributed to the activation of estrogen receptors α and β , as well as G-protein coupled or other membrane-associated estrogen receptors. Recently, we have identified a putative membrane-associated estrogen receptor that is coupled to desensitization of GABA_B receptors in guinea pig and mouse hypothalamic neurons including proopiomelanocortin (POMC) neurons. We have synthesized a new nonsteroidal compound, STX, which selectively targets the G α_q -coupled phospholipase C–protein kinase C–protein kinase A pathway, and have established that STX is more potent than E₂ in mediating this desensitization in an ICI 182, 780-sensitive manner in both guinea pig and mouse neurons. Both E₂ and STX are fully efficacious in estrogen receptor α , β knockout mice. Finally, we observed that the putative membrane-associated estrogen receptor is different from GPR30 in arcuate neurons using whole-cell patch recording in hypothalamic slices from GPR30 knockout mice. Collectively, these findings suggest that the mER is distinct from ER α , ER β or GPR30.

Keywords

G protein-coupled receptor; GABA_B receptor; potassium channels; POMC neurons GPR30

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1. Introduction

The hypothalamus controls multiple homeostatic functions that change dramatically during the ovulatory cycle and especially during pregnancy and menopause. Fluctuations in circulating estrogens are thought to be responsible for these changes in part through an action on hypothalamic neurons [1–4]. The hypothalamic proopiomelanocortin (POMC) neurons are target of estrogen and are critical neurons in the hypothalamic circuits controlling energy homeostasis [5,6]. POMC neurons through direct synaptic contacts modulate the excitability of hypothalamic neurons that control appetite, fluid balance, temperature, stress and reproduction [7]. In addition, POMC neurons project to other brain areas (e.g., midbrain) to control motivated behaviors such as sex and food consumption [8]. Although many of the actions of estrogen are mediated at the level of the hypothalamus, the cellular targets and signaling cascades involved in the effects of estrogen on homeostatic functions remain unclear.

Estrogens regulate cellular function through at least two signaling pathways broadly classified as “genomic” versus “rapid or nongenomic” [9,10]. In fact, both mechanisms can also lead to alterations in gene transcription. The genomic actions of estrogens in the hypothalamus and other brain areas have been attributed to the activation of estrogen receptor α (ER α) or ER β [9,11–13]. The “rapid” and “nongenomic” events are also believed to be mediated via ER α and ER β [14,15]; however, growing evidence suggests the involvement of plasma membrane receptors, especially G protein–coupled receptors (GPCRs). Potential candidates for novel membrane ERs include ER-X and the G-protein–coupled receptors, G α_q -mER and GPR30 [16–20].

In hypothalamic arcuate neurons including POMC neurons, we have identified a putative membrane-associated estrogen receptor (mER) that is G α_q -coupled to a phospholipase C–protein kinase C–protein kinase A (PLC–PKC–PKA) pathway [17,21]. The activation of this pathway by E₂ can rapidly dis-inhibit female arcuate neurons through desensitizing μ -opioid and GABA_B receptors. Recently, we have characterized the signaling of a nonsteroidal diphenylacrylamide compound, STX, that specifically targets the G-protein–coupled signaling pathway in both male and female arcuate neurons. Furthermore, we have established that E₂ and STX are fully efficacious in estrogen receptor knock-out (KO) mice. Therefore, this article will consider our current knowledge of rapid membrane-initiated and intracellular signaling by estrogen in the hypothalamic POMC neurons, the nature of receptors involved and how they contribute to homeostatic functions.

2. mER is a GPCR in hypothalamic POMC neurons

μ -Opioid and GABA_B receptors are linked to the same population of G-protein–coupled inwardly rectifying K⁺ (GIRK) channels in hypothalamic POMC, dopamine, and GABA neurons [22–24]. Activation of either μ -opioid or GABA_B receptors elicits an outward K⁺ current that robustly hyperpolarizes hypothalamic neurons. However, maximum activation of either receptor occludes the response of the other receptor [23,25,26]. Interestingly, short-term exposure to estrogen reduces the potency of both μ -opioid and GABA_B receptor agonists to activate GIRK channels in hypothalamic neurons [21,27,28]. The underlying

mechanism behind this estrogen-induced decrease in the responsiveness of hypothalamic neurons to the μ -opioid and GABA_B agonists has been elucidated over the past several years. Previous experiments have shown that selective PKA inhibitors can block the effects of estrogen and PKA activators mimic the effects of estrogen on the coupling of μ -opioid receptors to GIRK [21].

In recent experiments, we used the whole-cell recording method to measure the rapid effects of E₂ on the activation of the GIRK conductance by the GABA_B receptor agonist baclofen (Figure 1a) [17]. The pharmacokinetics of this rapid estrogen response further supported the involvement of a novel transmembrane estrogen receptor. The membrane-impermeable E₂-BSA conjugate gave an identical response to free E₂ suggesting that the hormone-binding site of this receptor is accessible from the extracellular surface of the plasma membrane. The E₂ response was stereospecific with respect to the configuration of the D-ring hydroxyl group; 17 β -estradiol (E₂) elicited the rapid response, whereas 17 α -estradiol was inactive. This is notable because 17 α -estradiol functions as an agonist of the nuclear ERs, albeit with reduced potency compared with 17 β -estradiol [29]. The selective estrogen receptor modulators (SERMs) 4-OH tamoxifen and raloxifene behaved like E₂ in mediating this response, whereas the steroidal anti-estrogen

ICI 182,780 antagonized the E₂ response. Moreover, we know that this membrane ER has a subnanomolar affinity for estrogen on the basis of our pharmacological (Schild) analysis [21]. Toran-Allerand et al. identified a high-affinity, saturable estrogen receptor, ERX, that is associated with caveolar-like microdomains in developing neocortical neurons [30]. This membrane-associated receptor is coupled to the activation of MAP kinases (MAPKs), extracellular-signal related kinase 1 (ERK1) and ERK2, which appear to be important for the development and survival of neurons [31–34]. Interestingly, ER-X also has a distinct pharmacology in that 17 α -estradiol is as equipotent as E₂ in activating the MAP kinase pathway [15,30]. However, we did not see any effects of 17 α -estradiol on the GABA_B response [17] or the μ -opioid response [21]. Similarly, Gu and Moss found that 17 α -estradiol did not mimic the actions of E₂ in the hippocampus to potentiate the glutamate (kainate)-mediated currents in CA1 pyramidal neurons [35]. Likewise, Mermelstein et al. found that 17 α -estradiol was much less efficacious than E₂ in reducing L-type calcium currents in neostriatal neurons [36]. Therefore, it appears that the mER that modulates channel activity in neurons via the PKC-PKA pathway is pharmacologically distinct from the receptor (ER-X) that is coupled to activation of ERK1 and ERK2 that promotes growth and survival.

A number of studies have shown that protein kinase pathways affect GABA_B receptor-mediated signaling in CNS neurons. Activation of protein kinase C suppresses the GABA_B receptor activation of GIRK channels in the hippocampal CA1 pyramidal neurons [37] and attenuates the GABA_B receptor-mediated inhibition of norepinephrine release from cerebellar slices [38]. In our experiments, we have found that the rapid GABA_B-suppressing effects of estrogen in hypothalamic neurons were sensitive to the broad-spectrum PKC inhibitor BIS but not to Gö6976, suggesting the involvement of a PKC not belonging to the conventional PKC class. In addition, the inhibition by estrogen of the GABA_B response was not altered by inclusion of 10 mM BAPTA in the intracellular recording patch pipette,

providing additional evidence that the Ca^{2+} -dependent conventional PKCs are not involved. However, the selective PKC δ inhibitor rottlerin blocked the actions of E_2 , suggesting that this novel-class PKC is a mediator of the rapid E_2 response. Moreover, our single cell RT-PCR data on the expression of PKC δ transcripts in arcuate neurons support the involvement of PKC δ in the E_2 -mediated inhibition of the GABA B response. PKC is also known to activate adenylyl cyclases [39–41]; moreover, when AC is activated by PKC instead of by G α s or forskolin, it is resistant to inhibition by G α i [42]. Notably, AC VII has a potential binding site for PKC δ that is not present in the sequences of the other adenylyl cyclases, which would allow PKC δ to directly phosphorylate AC VII [43]. In our study, we have shown that hypothalamic POMC neurons express AC VII transcripts [17]. Moreover, internal perfusion of BIS completely blocked the inhibition of the baclofen response by E_2 but did not attenuate the inhibition of the baclofen response by forskolin applied via bath perfusion, suggesting that PKC activation is upstream of PKA activation. Most PKCs are activated by diacylglycerol and some require the presence of Ca^{2+} . Thus, PKCs are downstream of the G α q-PLC-inositol triphosphate-diacylglycerol signaling cascade.

Our results show that this mER is specifically coupled to a G α q protein. This conclusion is based on experiments in which intracellular dialysis with a peptide fragment of G α q blocked the receptor interaction with G-protein. This G α q peptide has been used to block G α q signaling pathways in cortical pyramidal neurons [44]. In addition, the estrogen-mediated reduction of the GABA B response was significantly reduced by the phospholipase C inhibitor U73122 compared with cells perfused with the less active inhibitor U73343 suggesting that PLC is involved.

3. mER is distinct from ER α and ER β

The membrane-associated ERs might be derived from the same genes as ER α and ER β [45–48]. This belief stems primarily from the fact that many of the rapid effects of E_2 can be induced by selective ER α or ER β ligands, antagonized by the ER antagonist, ICI 182,780 or are lost in animals bearing mutations in ER α and/or ER β genes [15,46,49–52]. In ER α -deficient mice, rapid estrogen responses in certain regions of the mouse brain are lost [53], whereas the rapid action of estradiol in mouse hippocampal and arcuate neurons is not [18,54]. Thus, there remains conflicting indications of the role of ER α and/or ER β in rapid estrogen responses.

ER α is abundantly expressed in guinea pig arcuate neurons [55]. Also, ER β mRNA and protein are expressed in the rodent arcuate nucleus but to a much lesser extent than ER α [56–58]. Therefore, to verify that neither ER α nor ER β were involved in the rapid desensitization of the GABA B response by estrogen, we compared the effects of E_2 and STX [59]. In these studies, we characterized a mER that is G α q-coupled to a PLC–PKC–PKA pathway using STX as a ligand. Importantly, E_2 and STX were fully efficacious in activating this signaling pathway in both single and double-estrogen receptor knockout mice and had no proliferative effects on reproductive organs [18] (Fig. 1b and 1c). In fact, STX was a more selective ligand with even greater potency (~20-fold) for the mER [18]. Therefore, Our findings indicate that the mER in hypothalamic arcuate (POMC) neurons is distinct from ER α or ER β .

4. mER is not GPR30

Recently, different groups identified and characterized an orphan GPCR, GPR30, as a new estrogen-receptor involved in the rapid actions elicited by estrogen [60,61]. GPR30 has been reported to act independently of classical ERs to trigger rapid signaling by estrogens [61,62]. E₂ treatment of GPR30 transfected cells that lack expression of ER α and ER β caused activation of a stimulatory G protein (Gs) pathway that was directly coupled to this receptor, which led to an increase in AC activity [60]. Although GPR30 is thought to be a plasma membrane GPCR, it has been localized to endoplasmic reticulum and binds E₂ with nanomolar affinity [61]. Also, GPR30 has been identified in breast carcinoma cell lines that binds estrogen with relatively high affinity; however, the estrogen antagonist ICI 182,780 stimulates AC activity in SKBR3 cells that express GPR30, GPR30-transfected MDA-MB231 cells, and GPR30-transfected HEK (human embryonic kidney) cells [60,61,63], suggesting that GPR30 is different from mER in arcuate and neostriatal neurons [17,36].

Current work has found that GPR30 is also expressed in the brain, including the hypothalamus [20,64–66]. Within the hypothalamus, GPR30 mRNA is concentrated in nuclei such as the paraventricular, supraoptic, suprachiasmatic and arcuate as revealed using *in situ* hybridization [65]. Immunoreactive GPR30 is detected in the paraventricular and supraoptic nuclei of the hypothalamus, and in these regions GPR30 is expressed in oxytocin neurons [66,67]. Although GPR30 is localized in hypothalamic neurons, its pharmacology in hypothalamic neurons has not been characterized. We were interested in exploring whether GPR30 would be the “mER” that we have characterized in arcuate neurons. For this purpose we obtained GPR 30 knockout mice [68] and explored if E₂ would desensitize the GABA_B response in these animals as compared to wildtype. We found that E₂ was fully efficacious in inhibiting the GABA_B-induced effects in arcuate (POMC) neurons in GPR30 knockout mice (Fig. 2). This would suggest that GPR30 is not involved in this E₂-signaling pathway in arcuate neurons.

Therefore, from the collective results of several studies, we have formulated a signal transduction pathway for the rapid response to estrogen in arcuate neurons. The sequence of events in this model are as follows: (1) E₂ binds to a novel transmembrane estrogen receptor; (2) ligand binding activates G α_q ; (3) activated G α_q in turn activates PLC; (4) activated PLC hydrolyzes PIP₂ and liberates DAG; (5) free DAG stimulates PKC δ ; (6) PKC δ activates adenylyl cyclase (VII); (7) cAMP levels are elevated; (8) cAMP stimulates PKA; and (9) PKA phosphorylates membrane targets critical for K⁺ channel function [17].

5. Significance

It was known previously that E₂ could rapidly modulate synaptic efficacy via activation of PKA [21,35,69,70]. Recently, we have delineated the upstream components of this signaling pathway that includes G α_q , phospholipase C, and PKC δ activation. However, the exact identity of the membrane-associated estrogen receptor is still not known and many challenges lie ahead in fully identifying the nature of membrane steroid receptors. The physiological importance of this pathway is highlighted by the findings that food intake and body weight are influenced both by changes in endogenous estrogens and by exogenous

estrogenic treatments in mammals [71]. For example, Butera and Czaja [72] have shown that the anorexigenic effects of estrogen are attributable to the direct actions of the steroid in the arcuate-ventromedial hypothalamic nuclei. Our recent findings suggest that this is mediated, in part, via the hypothalamic mER. The physiological importance of the hypothalamic mER is further highlighted by the fact that systemically administered STX is effective in attenuating the weight gain after ovariectomy [18]. Moreover, in addition to its role in energy homeostasis, this hypothalamic mER may also be involved in temperature regulation, circadian rhythms, stress responses, and motivated behaviors.

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Figure 1.

E₂ and STX rapidly attenuated the baclofen response in hypothalamic arcuate (POMC) neurons in wild-type and ERαKO, ERβKO and ERαβKO mice. (a) Schematic drawing showing the protocol for drug administration in the whole-cell patch recording experiments. After seals were formed and the whole-cell configuration was obtained, slices were perfused with TTX (1 μM) for 5 min. The first baclofen response was generated by perfusing baclofen (at EC₅₀ concentration of 5 μM) until a steady-state outward current (first response, R₁) had been obtained (4–7 min). After drug discontinuation, the current returned to its predrug resting level. The cells were then treated with E₂ or other drugs for 15 min, and baclofen (5 μM) was perfused again, until finally a new maximum steady-state outward current (second response, R₂) was reached. The effect of estrogen on the baclofen response expressed as a percentage of R₂ over R₁. b, Bar graphs summarizing the effects of E₂ or STX in arcuate (POMC) neurons from ovariectomized, wild-type, ERαKO, and ERβKO mice. E₂ and STX (100 nM) rapidly attenuated the outward (GIRK) current induced by the GABA_B receptor agonist baclofen in arcuate (POMC) neurons from ovariectomized wild-type (C57BL/6), ERαKO, and ERβKO mice. Bars represent the mean ± SEM. ###p < 0.001, E₂-treated wild-type group versus vehicle (Veh) wild-type control; ***p < 0.001, E₂- or STX-treated ERαKO or ERβKO groups versus vehicle ERKO control. The data for the

ER α KO and ER β KO control responses were combined in one bar graph. c, Bar graphs summarizing the effects of E₂, STX, or ICI 182,780 in arcuate (POMC) neurons from male ER α β KO mice. E₂ and STX (100 nM) rapidly attenuated the outward (GIRK) current induced by the GABA_B receptor agonist baclofen, which was antagonized by ICI. ICI alone had no effect. Twelve of the 20 (60 %) neurons were identified as POMC neurons. Bars represent the mean $\pm$ SEM. ⁺⁺⁺p < 0.001, E₂- or STX- versus vehicle-treated cells from ER α β KO mice. [Reproduced from the Journal of Neuroscience 2006; 26:5649–5655 with permission from Society for Neuroscience]

Figure 2.

Estrogen rapidly attenuated the baclofen response in wild type and GPR30 knock-out mice

a. Two representative traces of the baclofen response in the presence of vehicle or E₂ (100 nM) in arcuate (POMC) neurons from GPR30 Knock-out mice [68] are shown. E₂ (100 nM) rapidly attenuated to baclofen (GABA_B) response by 40 %.

b. Bar graphs summarizing the effects of estrogen in arcuate (POMC) neurons from ovariectomized, wild type or GPR30 knock-out mice. 17β-estradiol (E₂) (100 nM) rapidly attenuated the outward (GIRK) current induced by the GABA_B receptor agonist baclofen in arcuate (POMC) neurons from ovariectomized wild type (C57BL/6) and GPR30 knockout mice. Bars represent the mean ± S.E.M. of 4–14 cells. ***p<0.005, E₂ treated group versus their vehicle control.